0647 - Neurite growth kinetics regulation through hydrostatic pressure in a novel triangle-shaped neurofluidic system.

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PROVIDING ORGANS-ON-CHIP FOR THE INDUSTRY

Introduction

Microfluidic applied to neuroscience research is based on the original model proposed by Taylor and colleagues, in which the connection between compartmentalized neuronal populations was achieved through guiding neurite outgrowth by using microchannels (Taylor *et al.*, 2005). This system has the advantage to:

- prevent the migration of cell bodies between culture channels while allowing **only neuronal projections to pass through** (Kanagasabapathi *et al.*, 2011),
- use the lenght of those microchannels to act as a **selectivity barrier** for the exclusive passage of axons over dendrites (Park *et al.*, 2006; Pan *et al.*, 2015),
- promote the **unidirectional neuronal projections** growth from one compartment to the other.

Nevertheless, it is crucial to develop microfluidic systems to control also neurite outgrowth dynamics.
Here, we introduce a **neurofluidic chip with a triangle-shaped design** that allows the exploration of neurite growth dynamics within asymmetric microchannels of various lengths (noted NF_2_X_TR).

Methodology

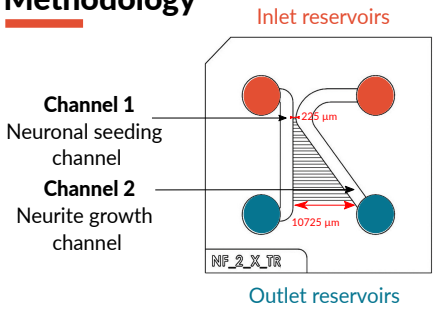

Fig. 1: Microfluidic device characteristics of NF_2_X_TR chip.

The device is made of two separated compartments, fluidically isolated, connected by microchannels of different lengths (Maisonneuve *et al.*, 2016). Neuronal bodies stay inside channel 1 and microchannels are narrow enough to only permit the passage of neurites into channel 2 in a unidirectional way.

Cultured cells (channel 1) were subjected to high pressure (HP) conditions:
- no addition of extra volume of medium (0 µL),
- addition of 40 µL of extra medium,
- addition of 80 µL of extra medium.

Cultured cells (channel 2) were subjected to negative pressure conditions:
- addition of 40 µL of extra medium,
- addition of 80 µL of extra medium.

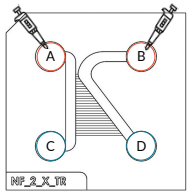

Fig. 2: Simulation of variable pressure conditions.

The induced HP was calculated from the applied variation of volume by using Bernoulli's principle: PB - PA = ρ x g x (VB/SB - VA/SA); P is the pressure at the chosen point, ρ is the density of the fluid at all points in the fluid, g is the acceleration due to gravity, V is the volume and S is the surface.

Results

1 Semi-automatic quantification of neurites growth dynamics

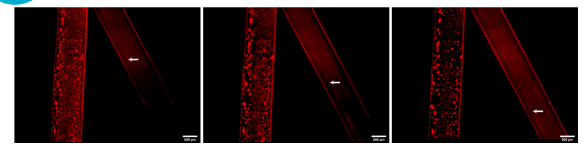

Fig. 3: Live-cell images of dissociated embryonic rats hippocampal cells at different time points, where the white arrows indicate the longest microchannel through which neurites extended.

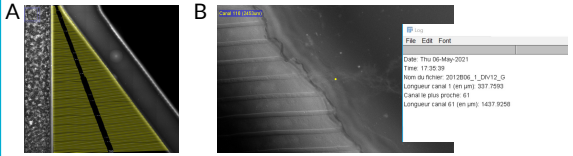

Fig. 4: Neurite length analysis using specific code. (A) Visualization of microchannels in NF_2_X_TR chip. (B) Example of results obtained with our analysis code.

The triangular 2-nodes chip allows to quantify the kinetic of neurites growth (Fig. 3).

We evaluate neurites length across several days *in vitro* using a proprietary software Fiji's script (Fig. 4) (Schindelin *et al.*, 2012).

This tool enables us to quantify the length of the microchannels after a manual selection of the longest neurite outgrowing in Channel 2.

2 Pressure influence neurites extension & orientation

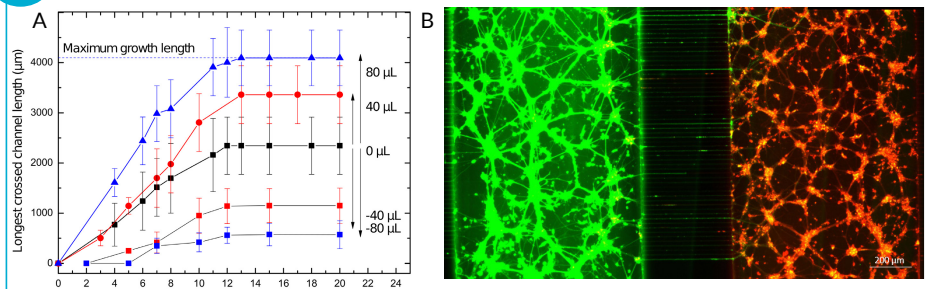

Fig. 5: (A) Evaluation of neurites growth dynamics according to different pressure condition (B) Standard microfluidic chip connected by microchannels, stainings with DiO (green) and DiD (red) solution

The maximum growth length is represented for the 80 µL hydrostatic condition.
A total of 80% of identified neurites grow from the compartment where the HP was positive towards the other compartment.

Conclusion and Perspectives

- Validation of a new method to enhance the maximum growth length and the guidance of neuronal projections into a specific direction using hydrostatic pressure instead of artificial chemical cues (Maisonneuve *et al.*, 2021).
- Efficient measurement of neurites outgrowth kinetics with a semi-automatic code.
- Suitable platform for studying the development of connections among compartmentalized microfluidic devices.
- Future work will focus on using such methodology to evaluate the effect of pharmacological compounds on neurites elongation and orientation on maturing and mature human culture post-neuronal injury.

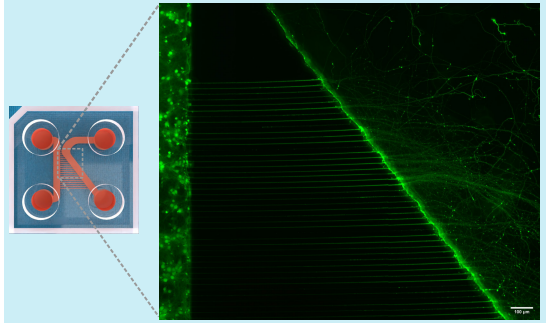

Fig.6: Immunofluorescence of rat hippocampal cell culture stained with anti-β-III tubulin in NF_2_X_TR device.

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